INFECTION AND IMMUNITY, Dec. 1987, p. 2945–2950
0019-9567/87/122945-06$02.00/0

Vol. 55, No. 12

Inhibition by 1,25(OH)$_2$-Vitamin D$_3$ of the Multiplication of Virulent Tubercle Bacilli in Cultured Human Macrophages

ALFRED J. CROWLE,* ELISE J. ROSS, AND MARY H. MAY

Webb-Waring Lung Institute and Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262

Received 27 April 1987/Accepted 26 August 1987

Historically, sunlight has seemed to fortify antituberculosis resistance. Evidence is presented here suggesting a role for vitamin D in this effect. The active metabolite of this photosynthesized vitamin, 1,25-dihydroxyvitamin D$_3$ (1,25D), promotes maturation and activation of human monocytes and macrophages (MPs). Therefore, it was tested for ability to protect MPs against virulent tubercle bacilli. MPs were derived by 7-day culture from blood monocytes, infected with the bacilli, and exposed to 1,25D in several regimens. Their inhibition of bacilli was measured by lysing samples of the cultures at 0, 4, and 7 days after infection and making bacillary CFU counts from serial dilutions of the lysates. 1,25D enabled MPs to slow or stop bacillary replication. Autologous serum supported the 1,25D-induced protection because the vitamin was not effective in medium supplemented with a serum substitute and was less effective in a heterologous AB serum than in autologous serum. The protection developed rapidly and could be induced even when 1,25D was added 3 days after infection. A concentration on the order of 4 μg/ml was needed for protection by the regimens used in these experiments. That is considerably higher than normal circulating concentrations of 1,25D but could be reached in infectious granulomas, because MPs can make 1,25D from precursor 25-hydroxyvitamin D$_3$. The precursor circulates at levels 10^3 higher than those of 1,25D and is directly influenced by dietary intake or photosynthetic production of vitamin D. These results identify 1,25D as an immunomodulator which can reproducibly activate human MPs to express tuberculoimmunity. They connect vitamin D, sunlight, and tuberculoimmunity and suggest that vitamin D should be considered a vital factor in the practical control of tuberculosis.

Vitamin D is photosynthesized, and sunlight seems to promote antituberculosis resistance (11, 15). Until recently, a connection between these two observations could be neither explained nor rigorously tested. However, the active form of the vitamin, 1,25-dihydroxyvitamin D$_3$ (1,25D), is now known to be an important immunoregulator (16). It modulates various monocyte, macrophage (MP), and lymphocyte activities (20), and it promotes human monocyte and MP maturation and activation (4, 16, 29). Tuberculoimmunity is expressed by activated MPs (3, 6) and consequently could be affected by 1,25D.

This possibility can now be directly tested in vitro with cultures of tubercle bacillus-infected human MPs (A. J. Crowle, *in* H. Friedman and M. Bendinelli, ed., *Tuberculosis: Interactions with the Immune System*, in press). In other experiments, this in vitro model of the host-parasite relationship in human tuberculosis has been used successfully to study native and acquired tuberculoimmunity (7, 12), bacillary virulence (8; Crowle, in press), and antituberculosis drugs (5, 9). This model provided important evidence that recombinant human gamma interferon does not protect human MPs against tubercle bacilli (14), which has recently been confirmed by Rook et al. (25) in a similar model.

This model therefore was used to investigate the effects of 1,25D on the human MP-tubercle bacillus relationship. The results reported here show that the vitamin can make MPs resistant to the bacilli. When integrated with other new knowledge of vitamin D, our findings lead to a reasonable explanation for the antituberculosis protectiveness of sunlight via this photosynthesized vitamin.

* Corresponding author.

MATERIALS AND METHODS

Media and ingredients. Human peripheral blood monocytes were purified on Ficoll-Hypaque and then washed and plated as fully described previously (7, 9, 13, 14). Briefly, a sample of heparinized blood was centrifuged on a layer of Ficoll-Hypaque (9% Ficoll 400 [Pharmacia, Piscataway, N.J.]; Hypaque, 50% diatrizoate sodium injection, USP [Winthrop-Breon, New York, N.Y.], diluted to 32% with distilled water). The isolated monocytes were washed four times and then suspended at 10^7 nucleated cells per ml in RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) for initial plating. The plating RPMI 1640 was supplemented with 2 mmol of L-glutamine, 52.6 μmol of sodium thioglycolate, and 1% unheated autologous serum; the culture RPMI 1640 was the same except that 50 U of potassium penicillin G was added per ml and the sodium thioglycolate was omitted. In some experiments, 10% serum substitute or 1% unheated AB serum was used instead of 1% autologous serum. Serum substitute is an ultrasonically stabilized mixture of 1.6 g of human serum albumin (fraction V, essentially fatty acid-free; no. A-1887; Sigma Chemical Co., St. Louis, Mo.), 0.4 mg of human transferrin (iron-free; no. T-2252; Sigma), 8 mg of cholesterol (99% grade; no. C-8253; Sigma), and 32 mg of L-α-phosphatidylcholine (no. P-6263; Sigma) in a volume of 40 ml of sodium bicarbonate-free Iscove medium (no. R 4140; Sigma) (see Stevenson et al. [28]). AB serum was obtained from a healthy male donor. Serum substitute was stored at 4°C, and sera were stored at −20°C.

1,25-Dihydroxycholecalciferol (Ro 21-5535; 5930-149) was a gift of Milan R. Uskokovic, Hoffmann-La Roche Inc., Nutley, N.J. It was kept as a stock solution of 1 mg in 1.5 ml

of 95% ethanol, stored at −20°C, and for use was diluted into the culture medium.

Recombinant human gamma interferon was a gift of Genentec, Inc. (San Francisco, Calif.). The original solution was diluted to 10^5 U/ml in physiologic phosphate buffer (pH 6.0), containing 5 mg of human serum albumin per ml, and stored at −90°C. For use, it was diluted in RPMI 1640, containing 50 mg of human serum albumin per ml, and then added to the culture medium.

Bacteriologic culture media (7H9 and 7H10) were bought from Difco Laboratories (Detroit, Mich.). Other materials used in these experiments were of standard laboratory grade.

Methods of culture and infection of human MPs. The following is a brief description of the culture and infection of MPs; full descriptions are published elsewhere (7, 9, 13; Crowle, in press).

Three 50-μl droplets of the freshly isolated monocytes were deposited separately per 35-mm petri dish (Falcon no. 1008, bacteriologic grade; Falcon Plastics, Oxnard, Calif.). After incubation for 30 min (incubations were at 37°C in 7.5% carbon dioxide in air), the nonadherent cells were washed off and discarded. The adherent cells were incubated for 7 days in 1.5 ml of culture medium per petri dish. These were then infected for 30 min at 37°C. Next, the infecting medium was drawn off, and the MPs were washed twice with unsupplemented RPMI 1640 and then cultured for up to 7 days more. Some plates were taken from each experiment after infection and at 4 and 7 days. Most were used for CFU counts of tubercle bacilli; others were fixed in glutaraldehyde and stained for counts of MPs and acid-fast bacilli.

Detailed characteristics of the MPs in these cultures are recorded elsewhere (Crowle, in press). Briefly, each monolayer spot averaged 5×10^4 MPs, of which 98% were viable by dye exclusion, 99% were positive for nonspecific esterase, and 100% could take up neutral red.

Bacteria and bacterial counts. The MP cultures were infected with virulent *Mycobacterium tuberculosis* Erdman prepared as detailed previously (9, 13). The bacillus/MP ratio for the 30-min period of infection in RPMI 1640 culture medium was 1:4, and at the end of infection about 20% of the MPs had taken up one to two culturable bacilli each (Crowle, in press).

The procedure for CFU counts is described in detail in reference 15. Sample plates were taken immediately after infection (0 time) and at 4 and 7 days. The organisms were lysed with sodium dodecyl sulfate, diluted in 7H9 medium, and plated at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} as quintuplet 20-μl droplets on 7H10 agar. For 4- and 7-day samples, culture supernatants were added to cell lysates to ensure counting of all CFU in each culture. Colonies of tubercle bacilli were counted on the 7H10 plates after 14 days of incubation. The data reported are CFU per milliliter of MP lysate, where 1 ml of lysate represents an average of 10^5 MPs. Generation times in hours for the growth of the intracellular bacilli were calculated by finding the average slope of each growth curve and then using that to measure from the graph the time required for CFU bacterial counts to double (see reference 8 for complete documentation).

The direct effect of 1,25D against tubercle bacilli in 7H9 medium was tested by adding it to bacillus-inoculated culture medium in the final concentrations indicated. This was done in triplicate in microtiter dishes as described previously for experiments with antimycobacterial drugs (10). The microtiter dishes were incubated for 7 days at 37°C. Readings of accumulated bacterial growth were made at 4 and 7

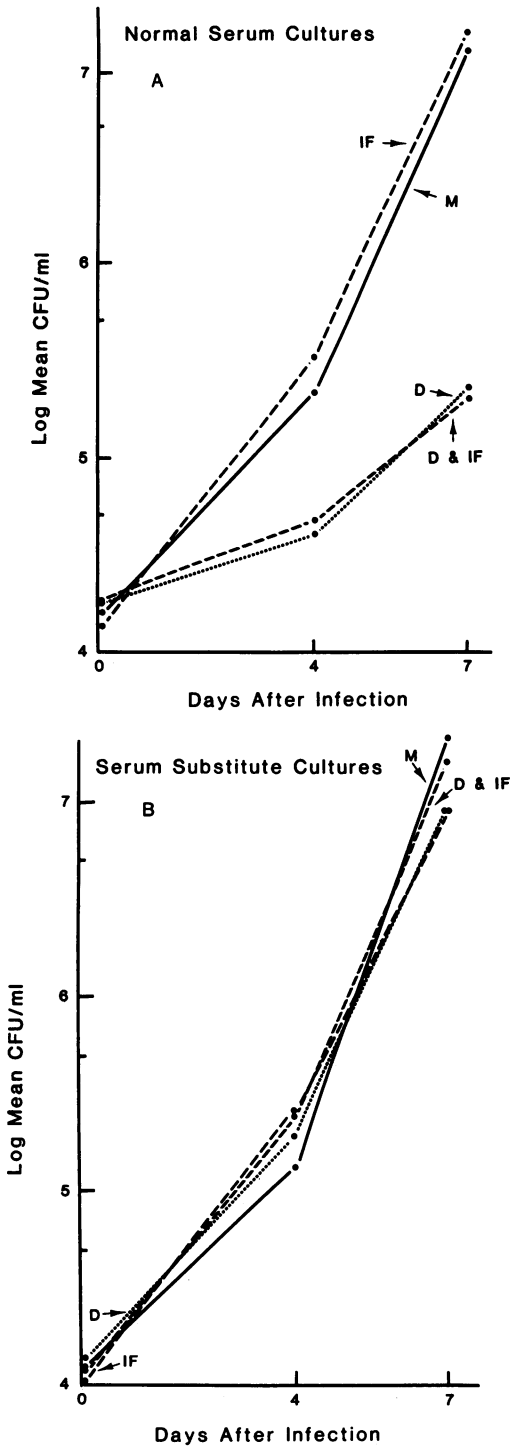

FIG. 1. Effects of 4 μg of 1,25D (D) per ml, 50 U of gamma interferon (IF) per ml, or both (D & IF), compared with medium alone (M), in medium supplemented with 1% normal autologous serum (A) or 10% serum substitute (B) on the growth of virulent tubercle bacilli in cultured human MPs over 7 days. The number of viable bacilli is plotted as mean CFU per milliliter of MP lysate (where 1 ml of lysate contained 10^5 MPs) versus days after infection, from samples taken immediately and at 4 and 7 days after infection. Each datum point is the mean of five values; standard errors of the means (not shown) usually were <10% of the means.

TABLE 1. Inhibition of virulent Erdman tubercle bacilli in cultured human MPs incubated in medium containing 4 μg of 1,25D per ml

Subject	Serum supplement[a]	Parameter (mean ± SEM)					
		Generation time[b] (h)		Ratio, G_D/G_C[c]	7-day CFU count (10^6)		Log_{10} difference, $CFU_C - CFU_D$[d]
		Control	1,25D		Control	1,25D	
All (11 subjects, 29 expts)	Normal	23.5 ± 0.98	71.8 ± 1.47	3.1	3.0 ± 0.19	0.2 ± 0.03	1.01 ± 0.18
Normal (5 subjects, 10 expts)	Normal	23.4 ± 1.8	71.3 ± 6.2	3.0	6.6 ± 3.9	0.2 ± 0.02	1.00 ± 0.18
Immune (6 subjects, 19 expts)	Normal	25.3 ± 0.8	74.2 ± 8.7	2.9	1.9 ± 0.2	0.1 ± 0.02	1.2 ± 0.12
8 Subjects, 9 expts	AB	24.6 ± 1.4	33.3 ± 2.7	1.4[e]	0.6 ± 0.16	0.3 ± 0.18	0.3 ± 0.12

[a] Medium was supplemented with normal or AB serum.
[b] Mean generation time of bacilli in cultured human MPs as determined by CFU counts (see the text).
[c] Mean generation time for the 1,25D groups (G_D)/mean generation time for the control groups (G_C).
[d] CFU_C, CFU of controls; CFU_D, CFU in 1,25D-treated cultures.
[e] In the nine experiments, the G_D/G_C ratio exceeded 2 in only one (2.1).

days on a 0 to 4+ scale, where 4+ was the amount of bacterial growth in medium-only control wells at 7 days.

MP donors. MPs were obtained from five normal and six immunized Caucasian volunteers, male and female, ranging in age from the late 20s to the mid-50s (informed consent obtained; race specified because of differences in vitamin D photosynthesis among the races; see reference 1). The immunized donors had received either commercial *Mycobacterium bovis* BCG or a trypsin-extracted experimental immunizing antigen (9). The five normal donors had no history of tuberculin skin test positivity or contact with tuberculosis.

EXPERIMENTS AND RESULTS

Comparative protection by 1,25D alone versus gamma interferon or the two combined. The protectiveness of 1,25D alone against tubercle bacilli in cultured human MPs was discovered in experiments attempting to demonstrate an antimycobacterial immune lymphokine function for gamma interferon by using 1,25D as a potential synergistic activator (Fig. 1). The replication of tubercle bacilli in normal MPs in medium, gamma interferon, 1,25D, or a combination of 1,25D and gamma inteferon was compared. In half the experiment, the culture medium was supplemented with 1% autologous serum, and in the other half 10% serum substitute was used. One day before infection, and also immediately after infection, 50 U of gamma interferon per ml, 4 μg of 1,25D per ml, or both were added to the medium in comparison with medium alone. The 50-U/ml dose and regimen of gamma interferon were chosen from previous experiments as the strongest for activating human MPs for cytotoxic and antileishmanial immunity without being toxic for tubercle bacillus-infected MP (14, 22).

1,25D alone was highly protective in medium supplemented with 1% autologous serum, but gamma interferon was not (Fig. 1A). Neither 1,25D nor gamma interferon was protective in medium supplemented with 10% serum substitute (Fig. 1B).

Consistent protection by 1,25D alone. The regimen of 4 μg of 1,25D per ml, added to the culture medium supplemented with 1% autologous serum at 24 h before infection and also after infection, was confirmed protective in a series of 29 experiments testing MPs and sera from five normal subjects and six immune subjects (Table 1). It caused an average threefold slowing of intra-MP bacillary replication (mean generation time of 23.5 h, diminishing to a mean of 71.8 h) equally in MPs from the two different kinds of subjects. It effected an average 10-fold suppression of CFU counts per MP accumulated by the end of the 7-day period of infection.

In several of these experiments, results from the CFU counts were confirmed by back-up acid-fast bacillus counts. The numbers of MPs in these cultures usually diminished, similarly in all groups, by about 10% during the period of infection (a consistent characteristic of this model; 13, 14; Crowle, in press). The morphology of MPs in the presence of 1,25D varied among donors. Usually, they looked "activated" (enlarged, spread, and firmly adherent, with ruffled membrane and pale nucleus with prominent nucleoli), but sometimes they were rounded and poorly attached. Whichever was the case, more than 99% were viable, by dye exclusion test, at the end of the period of infection. No consistent correlation between a predominating morphologic appearance in these cultures and expression of antibacillary resistance was noted.

Diminished protectiveness of 1,25D in AB serum-supplemented medium. Several experiments confirmed that this regimen of 1,25D was not protective in medium supplemented with 10% serum substitute (data not shown, but see Fig. 1B). As an alternative "neutral" supplement, 1% AB serum was used in several experiments. Table 1 shows results from nine of these experiments, using MPs from eight subjects. 1,25D was less protective in the AB serum than in normal serum.

Concentration of 1,25D required for protection. Concentrations of 1,25D at 4 μg/ml and less in 10-fold steps were tested on 6-day and 4-day MPs and on just-plated (0-day) adherent monocytes. Concentrations higher than 4 μg/ml were not practical because of the ethanol used to dissolve the 1,25D. The 0- and 4-day cells received 1,25D only once, after infection; the 6-day cells received it at both 24 h before infection and after infection. Results from three experiments, two with 6-day MPs and one with the 4-day MPs or 0-day monocytes, are summarized in Table 2. They show good protection at 4 μg/ml but little or none at lower concentrations. These data, which are also graphed in Fig. 2 for the 6-day MPs, also show that 1,25D protectiveness began soon after addition of the 1,25D, tended to grow stronger over several days, and could become strong enough for bacteriostasis.

Protectiveness of single-addition 1,25D. Protection by single-addition 1,25D (Table 2) indicated that the previously mostly used two-addition regimen (e.g., Table 1) was unnecessary. This was confirmed in an experiment comparing two additions (24 h before infection and after infection) with one addition of the 1,25D at different times to the MP culture medium. The results (Fig. 3) indicated that the vitamin,

when added solely after infection, was as protective as when added twice. It was also protective when its addition was delayed to 3 days after infection, but not when it was added once at 3 days before infection.

Direct effect of 1,25D on tubercle bacilli. The direct effect of 1,25D on tubercle bacilli was tested by adding the vitamin to Erdman-inoculated 7H9 bacteriologic culture medium, or to this medium containing 1% normal human serum or 10% serum substitute, and incubating these cultures for 7 days with readings at 4 and 7 days. Inoculated media similarly received concentrations of ethanol equivalent to those used to dissolve the 1,25D. Growth of the bacilli was not inhibited in any of the media by 1,25D at up to 40 μg/ml, i.e., 10 times greater than used in the MP cultures, or in the ethanol solvent.

DISCUSSION

Tuberculoimmunity is supposed to be expressed by lymphokine-activated MPs (3, 6). Gamma interferon is a lymphokine which can protect human MPs against various parasites (27) and some intracellular bacteria (2), but apparently not tubercle bacilli (14, 25). This nonprotectiveness was reconfirmed here (Fig. 1).

Attempts to use 1,25D to help gamma interferon protect the MPs showed that 1,25D alone was protective. This was a reproducible finding, being confirmed in a series of 29 experiments with MPs from both normal and immunized donors (Table 1).

TABLE 2. 1,25D concentration needed to protect cultured human monocytes or MPs against virulent Erdman tubercle bacilli[a]

Responding cells and 1,25D concn (μg/ml)	Generation time (h)	Ratio, G_D/G_C[b]	7-Day CFU
0-day monocytes			
0.0	20.7	1.0	1.7×10^6
4.0	30.9	1.5	1.3×10^5
0.4	23.2	1.1	9.2×10^5
0.04	20.8	1.0	1.5×10^6
0.004	21.6	1.0	9.6×10^5
4-day MPs			
0.0	23.3	1.0	7.3×10^5
4.0	43.0	1.9	7.4×10^4
0.4	32.8	1.4	1.7×10^5
0.04	28.3	1.2	3.0×10^5
0.004	27.4	1.2	2.6×10^5
6-day MPs			
0.0	25.5	1.0	1.4×10^6
4.0	117.9	4.6	1.8×10^4
0.4	24.4	1.0	2.3×10^6
0.04	24.0	0.9	1.7×10^6
0.004	22.0	0.9	1.4×10^6
Ethanol[c]	26.9	1.1	1.4×10^6
4.0 (once)[d]	52.0	2.0	1.1×10^5

[a] Adherent cells were infected immediately after isolation as 0-day monocytes. They received 1,25D only once, immediately after infection. The 6-day MPs received 1,25D at the concentrations indicated twice, at 24 h before and immediately after infection; the 0- and 4-day cells were treated just once, immediately after infection.

[b] Mean generation time for the 1,25D groups (G_D)/mean generation time for the control groups (G_C).

[c] Ethanol was added to the medium at a final concentration of 0.6%, to be equivalent to the concentration of ethanol in the medium of the other groups where it was added as the solvent for the 1,25D when the 1,25D was used at 4 μg/ml.

[d] 1,25D was added immediately after infection only for comparison of the effect on the 0-day monocytes, which also received it only once.

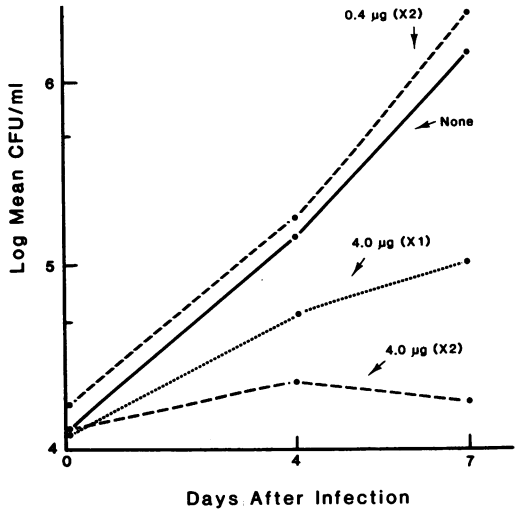

FIG. 2. Comparison of MP protection against tubercle bacilli of 4 or 0.4 μg of 1,25D per ml, given at both 24 h before and immediately after infection (×2), and of 4 μg/ml given only once immediately after infection (×1).

A serum cofactor appeared to be necessary, because the protectiveness of 1,25D seen in 1% unheated autologous serum was lost in medium alternatively supplemented with a serum substitute (Fig. 1). The vitamin was also less protective when AB serum was used in place of autologous serum (Table 1), possibly suggesting some genetic restriction for the cofactor. The nature of this cofactor is unknown.

1,25D induced resistance in the MPs rapidly (Fig. 2). Over the next several days, the resistance usually appeared to grow stronger. However, this appearance may actually have been due to slowly cumulative damage to the intracellular tubercle bacilli. The two-addition regimen (24 h before infection and then also immediately after infection), used in most of the experiments, was not necessary. Single addition of the 1,25D after infection also was protective (Fig. 3, Table

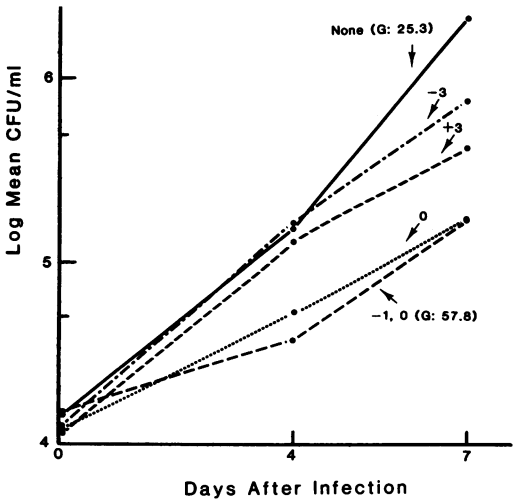

FIG. 3. Effects of 4 μg of 1,25D per ml on tubercle bacillus growth in MPs when added to the culture medium once at 3 days before (−3), immediately after (0), or 3 days after (+3) infection, compared with both at 1 day before and immediately after (−1, 0). G, Mean generation time (in hours) of bacilli in the MP cultures.

2). Protection either was not induced or was lost if the 1,25D was added to MP cultures once at 3 days before infection.

Concentrations of 1,25D near 4 μg/ml were needed for good protection. These seem unphysiologically high compared with the 26 to 70 pg/ml in the normal circulating range (27). One explanation for this could be immunologically inefficient use of the 1,25D. Much smaller concentrations used in other regimens are able to promote maturation and activation of human monocytes (21), especially in synergy with other immunomodulatory molecules (20). However, the high concentration that was directly protective in our experiments might actually be reached in MP-rich granulomas as discussed below, because 1,25D can be synthesized by MPs (23, 24; see also 26).

The 1,25D was able to protect MPs strongly enough in some experiments to stop intracellular multiplication of highly virulent Erdman strain tubercle bacilli (Fig. 2). The protection probably was an induced response of the MPs. An alternative possibility, that it was due to killing of the MPs by either the 1,25D or the ethanol it was dissolved in, was excluded by regular confirmation of normal MP health and numbers and of the suppressed growth of the bacilli in the MPs by acid-fast-bacillus counts. Direct inhibition of the bacilli by the 1,25D seems unlikely, because neither the vitamin nor the ethanol solvent inhibited the bacilli directly at concentrations up to 10 times higher than used in the MP cultures. The requirement of a serum cofactor supports the theory of action via the MPs. Furthermore, it is unlikely from what is known about transportation of 1,25D through MPs and its affinity for the nucleus (16–18, 29) that the 1,25D would have much contact with the phagosomally confined (Crowle, in press) bacilli.

While the experiments reported here were being done, Rook et al. (26) published similar results showing that 1,25D can protect cultured human monocytes against tubercle bacilli. Thus, completely independent findings from two different laboratories using somewhat different techniques and assays now provide agreeing direct evidence for a mechanism of antituberculosis protection, via the vitamin D, fitting several centuries (11, 15) of observations of the protectiveness of sunshine against tuberculosis. These findings suggest that vitamin D may be necessary for expressing tuberculoimmunity. If so, many people may be unduly susceptible to tuberculosis from lack of vitamin D owing to indifference to dietary supplementation and insufficient sunlight (1, 11, 19). A modern analysis of this possibility and some epidemiologic evidence for it were published recently by Davies (11).

ACKNOWLEDGMENTS

This work was supported by grants from the Robert J. Kleberg, Jr., and Helen C. Kleberg Foundation, the IMMTUB program of the World Health Organization, the Maytag-Crawford Trust, the Potts Foundation, and the Pittsfield Antituberculosis Association.

LITERATURE CITED

1. **Belton, N. R.** 1985. Vitamin D deficiency in Asian children in Britain—a case for prophylactic supplementation?, p. 579–580. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.
2. **Bhardwaj, N., T. W. Nash, and M. A. Horwitz.** 1986. Interferon-gamma-activated human monocytes inhibit the intracellular multiplication of *Legionella pneumophila*. J. Immunol. **137**:2662–2669.
3. **Chaparas, S. D.** 1982. The immunology of mycobacterial diseases. Crit. Rev. Microbiol. **9**:139–197.
4. **Cohen, M. S., D. E. Mesler, R. G. Snipes, and T. K. Gray.** 1986. 1,25-Dihydroxyvitamin D_3 activates secretion of hydrogen peroxide by human monocytes. J. Immunol. **136**:1049–1053.
5. **Crowle, A. J.** 1986. Studies of antituberculosis chemotherapy with an in vitro model of human tuberculosis. Semin. Respir. Infect. **1**:262–264.
6. **Crowle, A. J., G. S. Douvas, and M. H. May.** 1983. The cellular and molecular nature of human tuberculoimmunity. Bull. Int. Union Tuberc. **58**:72–80.
7. **Crowle, A. J., and M. May.** 1981. Preliminary demonstration of human tuberculoimmunity in vitro. Infect. Immun. **31**:453–464.
8. **Crowle, A. J., and M. H. May.** 1983. Replication of lyophilized and cultured BCG in human macrophages. Am. Rev. Respir. Dis. **128**:673–679.
9. **Crowle, A. J., J. A. Sbarbaro, F. N. Judson, G. S. Douvas, and M. H. May.** 1984. Inhibition by streptomycin of tubercle bacilli within cultured human macrophages. Am. Rev. Respir. Dis. **130**:839–844.
10. **Crowle, A. J., J. A. Sbarbaro, F. N. Judson, and M. H. May.** 1985. The effect of ethambutol on tubercle bacilli within cultured human macrophages. Am. Rev. Respir. Dis. **132**:742–745.
11. **Davies, P. D. O.** 1985. A possible link between vitamin D deficiency and impaired host defence to *Mycobacterium tuberculosis*. Tubercle **66**:301–306.
12. **Douvas, G. S., E. M. Berger, J. E. Repine, and A. J. Crowle.** 1986. Natural mycobacteriostatic activity in human monocyte-derived adherent cells. Am. Rev. Respir. Dis. **134**:44–48.
13. **Douvas, G. S., and A. J. Crowle.** 1985. Macrophages. I. Model for studying antimicrobial immunity in vitro, p. 81–90. *In* T. Yoshida (ed.), Investigation of cell-mediated immunity. Churchill Livingstone, New York.
14. **Douvas, G. S., D. L. Looker, A. E. Vatter, and A. J. Crowle.** 1985. Gamma interferon activates human macrophages to become tumoricidal and leishmanicidal but enhances replication of macrophage-associated mycobacteria. Infect. Immun. **50**:1–8.
15. **Goldberg, G.** 1946. Clinical tuberculosis, 5th ed., p. E3–E45. F. A. Davis Co., Philadelphia.
16. **Gray, T. K., and M. S. Cohen.** 1985. Vitamin D, phagocyte differentiation and immune function. Surv. Immunol. Res. **4**:200–212.
17. **Hassad, J. G.** 1985. Plasma vitamin D binding protein-actin interactions: fact and artifact, p. 682–688. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.
18. **Haussler, M. R., C. A. Donaldson, M. A. Kelly, D. J. Mangelsdorf, and S. L. Marion.** 1985. Functions and mechanism of action of the 1,25-dihydroxyvitamin D_3 receptor, p. 83–92. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.
19. **Holick, M. F.** 1986. Sunlight and skin: their role in vitamin D nutrition for humans, p. 15–43. *In* D. A. Roe (ed.), Nutrition and the skin. Alan R. Liss, Inc., New York.
20. **Manolagas, S. C.** 1985. Role of 1,25-dihydroxyvitamin D_3 in the immune system, p. 199–208. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.
21. **Ohta, M., T. Okabe, K. Ozawa, A. Urabe, and F. Takaku.** 1986. In vitro formation of macrophage-epithelioid cells and multinucleated giant cells by $1,25(OH)_2D3$ from human circulating monocytes. Ann. N.Y. Acad. Sci. **465**:211–220.
22. **Passwell, J. H., R. Shor, and J. Shoham.** 1986. The enhancing effect of interferon-beta and -gamma on the killing of *Leishmania tropica major* in human mononuclear phagocytes in vitro. J. Immunol. **136**:3062–3066.
23. **Reichel, H., H. P. Koeffler, R. Bargers, R. Munker, and A. W. Norman.** 1985. 1,25-Dihydroxyvitamin D_3 and the hematopoietic system, p. 167–176. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.

24. **Reichel, H., H. P. Koeffler, J. E. Bishop, and A. W. Norman.** 1987. 25(OH)vitamin D_3 metabolism by lipopolysaccharide-stimulated normal human macrophages. J. Clin. Endocrinol. & Metabol. **64**:1–9.
25. **Rook, G. A. W., J. Steele, M. Ainsworth, and B. R. Champion.** 1986. Activation of macrophages to inhibit proliferation of *Mycobacterium tuberculosis*: comparison of the effects of recombinant gamma-interferon on human monocytes and murine peritoneal macrophages. Immunology **59**:333–338.
26. **Rook, G. A. W., J. Steele, L. Fraher, S. Barker, R. Karmali, J. O'Riordan, and J. Stanford.** 1986. Vitamin D_3, gamma interferon, and control of proliferation of *Mycobacterium tuberculosis* by human monocytes. Immunology **57**:159–163.
27. **Schwartzman, M. S., and W. A. Frank.** 1987. Vitamin D toxicity complicating the treatment of senile, postmenopausal, and glucocorticoid-induced osteoporosis. Am. J. Med. **82**:224–230.
28. **Stevenson, H. C., E. Schlick, R. Griffith, M. A. Chirigos, R. Brown, J. Conlon, D. J. Kanapa, R. K. Oldham, and P. Miller.** 1984. Characterization of biological response modifier release by human monocytes cultured in suspension in serum-free medium. J. Immunol. Methods **70**:245–256.
29. **Teitelbaum, S. L., Z. Bar-Shavit, P. H. Reitsma, H. G. Welgus, and A. J. Kahn.** 1985. Vitamin D and macrophage differentiation, p. 177–182. *In* A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. Herath (ed.), Vitamin D: chemical, biochemical, and clinical update. Walter de Gruyter, Inc., New York.